Mood and autonomic responses to repeated exposure to the Trier Social Stress Test for Groups (TSST-G)

Bösch, Maria ; Sefidan, Sandra ; Ehlert, Ulrike ; Annen, Hubert ; Wyss, Thomas ; Steptoe, Andrew ; La Marca, Roberto

Abstract: Introduction A group version of the Trier Social Stress Test (TSST-G) was introduced as a standardized, economic and efficient tool to induce a psychobiological stress response simultaneously in a group of subjects. The aim of the present study was to examine the efficacy of the TSST-G to repeatedly induce an affective and autonomic stress response while comparing two alternative protocols for the second examination. Methods and material Healthy young male recruits participated twice in the TSST-G 10 weeks apart. In the first examination, the TSST-G consisted of a combination of mental arithmetic and a fake job interview (TSST-G-1st; n = 294). For the second examination, mental arithmetic was combined with either (a) a defensive speech in response to a false shoplifting accusation (TSST-G-2nd-defence; n = 105), or (b) a speech on a more neutral topic selected by the investigators (TSST-G-2nd-presentation; n = 100). Affect ratings and salivary alpha-amylase (sAA) were determined immediately before and after the stress test, while heart rate (HR) and heart rate variability (HRV) were measured continuously. Results TSST-G-1st resulted in a significant increase of negative affect, HR, and sAA, and a significant decrease in positive affect and HRV. TSST-G-2nd, overall, resulted in a significant increase of HR and sAA (the latter only in response to TSST-G-2nd-defence) and a decrease in HRV, while no significant affect alterations were found. When comparing both, TSST-G-2nd-defence and -2nd-presentation, the former resulted in a stronger stress response with regard to HR and HRV. Discussion The findings reveal that the TSST-G is a useful protocol to repeatedly evoke an affective and autonomic stress response, while repetition leads to affective but not necessarily autonomic habituation. When interested in examining repeated psychosocial stress reactivity, a task that requires an ego-involving effort, such as a defensive speech, seems to be significantly superior to a task using an impersonal speech.

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Author: Maria Boesch Sandra Sefidan Ulrike Ehlert Hubert Annen Thomas Wyss Andrew Steptoe Roberto La Marca

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Mood and autonomic responses to repeated exposure to the Trier Social Stress Test for Groups (TSST-G)

Running title: Repeated exposure to the Trier Social Stress Test for Groups

Authors:

Maria Boesch[1, 2], Sandra Sefidan[1, 2], Ulrike Ehlert[1], Hubert Annen[2], Thomas Wyss[3], Andrew Steptoe[4], & Roberto La Marca[1]

Affiliations:

[1]Clinical Psychology and Psychotherapy, University of Zurich, Zurich, Switzerland

[2]Military Academy, Swiss Federal Institute of Technology ETH Zurich, Zurich, Switzerland

[3]Swiss Federal Institute of Sports Magglingen SFISM, Magglingen, Switzerland

[4]Department of Epidemiology and Public Health, University College London UCL, London, United Kingdom

Corresponding author:

Name:	Roberto La Marca, Ph.D.
Mailing address:	Department of Clinical Psychology and Psychotherapy University of Zurich Binzmuehlestrasse 14/Box 26 CH-8050 Zurich
e-mail:	r.lamarca@psychologie.uzh.ch
Telephone:	+41 44 635 73 62
Fax:	+41 44 635 73 59

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SUMMARY

Introduction: A group version of the Trier Social Stress Test (TSST-G) was introduced as a standardized, economic and efficient tool to induce a psychobiological stress response simultaneously in a group of subjects. The aim of the present study was to examine the efficacy of the TSST-G to repeatedly induce an affective and autonomic stress response while comparing two alternative protocols for the second examination.

Methods and material: Healthy young male recruits participated twice in the TSST-G 10 weeks apart. In the first examination, the TSST-G consisted of a combination of mental arithmetic and a fake job interview (TSST-G-1st; n = 294). For the second examination, mental arithmetic was combined with either (a) a defensive speech in response to a false shoplifting accusation (TSST-G-2nd-defence; n = 105), or (b) a speech on a more neutral topic selected by the investigators (TSST-G-2nd-presentation; n = 100). Affect ratings and salivary alpha-amylase (sAA) were determined immediately before and after the stress test, while heart rate (HR) and heart rate variability (HRV) were measured continuously.

Results: TSST-G-1st resulted in a significant increase of negative affect, HR, and sAA, and a significant decrease in positive affect and HRV. TSST-G-2nd, overall, resulted in a significant increase of HR and sAA (the latter only in response to TSST-G-2nd-defence) and a decrease in HRV, while no significant affect alterations were found. When comparing both, TSST-G-2nd-defence and -2nd-presentation, the former resulted in a stronger stress response with regard to HR and HRV.

Discussion: The findings reveal that the TSST-G is a useful protocol to repeatedly evoke an affective and autonomic stress response, while repetition leads to affective but not necessarily autonomic habituation. When interested in examining repeated psychosocial stress reactivity, a task that requires an ego-involving effort, such as a defensive speech, seems to be significantly superior to a task using an impersonal speech.

Keywords: Trier Social Stress Test for Groups; repeated stress provocation; autonomic nervous system; salivary alpha-amylase; heart rate; heart rate variability

Abbreviations: ANS: autonomic nervous system; BL: baseline; HPA axis: hypothalamic-pituitary-adrenal axis; HR: heart rate; HRV: heart rate variability; Intro: introduction; PANAS: positive and negative affect schedule; Rec: recovery; RMSSD: square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals; sAA: salivary alpha-amylase; S1: speech task; S2: mental arithmetic task; TSST(-G): Trier Social Stress Test (for Groups); TSST-G-1st: first exposure to TSST-G including both subgroups (defence and presentation); TSST-G-2nd-defence: second exposure to TSST-G with a defensive speech; TSST-G-2nd-presentation: second exposure to TSST-G with speech on a more neutral topic

INTRODUCTION

Stress is an omnipresent phenomenon in everyday life, and it is associated with alterations of biological variables. Pathophysiologic mechanisms are thought to constitute a major pathway through which stress leads to the development or aggravation of some mental disorders (e.g. anxiety disorders, depression) as well as somatic diseases (e.g. cardiovascular disease, type 2 diabetes mellitus; Chrousos, 2009). Health-related research often focuses on changes in physiologic stress systems (e.g. to examine the effects of a treatment or intervention). Therefore, examination of changes in psychobiological stress reactivity requires the application of valid repeatable stress provocation tools. Since factors such as novelty and unpredictability are important determinants of stress provocation (Dickerson & Kemeny, 2004), repeated stress exposure poses a challenge for the correct choice of a stressor and makes interpretation of response alterations difficult.

Kirschbaum et al. (1993) developed a standardized stress-provoking procedure, the Trier Social Stress Test (TSST), inducing a psychobiological stress response in humans. The original TSST consists of a free speech and a mental arithmetic task (serial subtraction) in front of an audience. Extensive evidence supports the effectiveness of the TSST in inducing a multidimensional stress response (Dickerson & Kemeny, 2004; Campbell & Ehlert, 2012). Two major physiologic stress systems, the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) with its sympathetic and parasympathetic branch, have repeatedly been shown to respond to the TSST acutely. While biomarkers like cortisol (Schoofs & Wolf, 2011; Hellhammer & Schubert, 2012), salivary alpha-amylase (sAA) (Nater et al., 2006; Schoofs & Wolf, 2011), and heart rate (HR) (Nater et al., 2005; Hellhammer & Schubert, 2012) increase, heart rate variability (HRV) decreases in response to the TSST (Nater et al., 2006; Strahler et al., 2010). Taken together, the TSST is a powerful psychosocial stress task inducing an acute stress response of all main physiologic stress systems.

To maintain the efficacy of the TSST in provoking stress-induced responses with repeated applications, some changes to the stress protocol are advised. To avoid participants presenting a memorized speech or remembering the sequence of correct responses to the mental arithmetic task on the second assessment session, different adaptations of the TSST subtasks have been used in the past: While during the first exposure, the original protocol of the TSST (Kirschbaum et al., 1993) is often applied, during the second exposure the job interview scenario is slightly adapted (Kirschbaum et al., 1995; Schommer et al., 2003; Petrowski et al., 2012) or replaced by a defensive speech in response to a false shoplifting accusation (Saab et al., 1989; Burleson et al., 2003). For the mental arithmetic subtask, most authors change the initial number of the serial subtraction task for the second exposure (Kirschbaum et al., 1995; Schommer et al., 2003; Petrowski et al., 2012). Repeated exposure to the TSST has produced different changes in various physiological stress systems. The cortisol response usually habituates to repeated exposure to minimally changed stress tasks (Kirschbaum et al., 1995; Schommer et al., 2003; Wüst et al., 2005; Kudielka et al., 2006), whereas the stress response of sAA (Gerra et al., 2001; Schommer et al., 2003; von Känel et al., 2006), HR (Gerra et al., 2001; Schommer et al., 2003; von Känel et al., 2006; Jönsson et al., 2010) and HRV (Petrowski et al., 2012) show very little habituation to repeated stress exposure.

Even though the TSST is very effective, it requires significant resources in terms of staff and, therefore, financial means. Much effort is required for each individual participant. In case of repetition, twice the resources are needed. Therefore, more economic stress test procedures are desirable, especially when resources are scarce or subject samples are large. In 2006

Childs et al. applied, for the first time, a method of administering the TSST in a group setting. Individual persons and groups of two or three healthy volunteers were simultaneously monitored for salivary cortisol and HR responses to the TSST, with significant results in both parameters, and HR showed an even higher peak in the group setting compared to the single setting. Subsequently, the new protocol of the Trier Social Stress Test for Groups (TSST-G) was evaluated and published by von Dawans et al. (2011). These authors examined groups of six participants and included, additionally, a specific control condition (a single-blind control condition containing all factors except the psychosocially stressful components, i.e. socio-evaluative threat and uncontrollability). Von Dawans et al. (2011) replicated and extended the findings presented by Childs et al. (2006). In fact, they found that the TSST-G induced significant increases in salivary cortisol and HR. In comparison with the control condition, the TSST-G produced larger stress reactions in both parameters. Until now no study has examined whether the application of the TSST-G also provokes sAA and HRV changes, and whether a repetition of the TSST-G can effectively induce a biopsychological stress response.

Therefore we designed the study to test the hypothesis that the TSST-G is an effective stress task in terms of the autonomic system. On the other hand, the hypothesis was explorative to test the efficacy of a repeated exposure to the TSST-G with an interval of 10 weeks, while comparing two adaptations of the TSST-G during the second exposure.

METHODS AND MATERIAL

Participants and Procedure

This investigation was part of a larger project on mental and physical health during basic military training of Swiss Armed Forces. The project was approved by the Ethics Committee of the Canton of Aargau. Switzerland has a compulsory military service for men. After a complex recruitment procedure, consisting of physical examinations (e.g. eye test, ear test, electrocardiogram, pulmonary function test, and analyses of blood) and psychological screening questionnaires (e.g. depression), every young man who is rated to be physically and psychologically healthy is approved for military service. Therefore, these recruits consisted of a representative sample of healthy young Swiss men. During the first week of basic military training in the second part of 2011 subjects were recruited (overall: $n = 694$). After a briefing on the study, volunteering recruits provided signed consent ($n = 651$). Inclusion criteria were German speaking (Switzerland is a multilingual country) and male sex ($n = 569$). Exclusion criteria were Italian or French speaking ($n = 81$) and female sex ($n = 1$). Due to time and electrophysiological device restrictions, 302 recruits were randomly selected from the pool of potential subjects to participate in the psychobiological examinations, while the rest completed only questionnaires. The selected subjects participated in two stress examinations: Once during the 1st week and once during the 11th week of military service. During the second examination, two subgroups were assessed to test slightly different adaptations of the TSST-G (see below). Because recruits were divided into two equivalent units by this time, allocation to the TSST-G subgroups was defined by company membership. The psychobiological examinations were embedded in an ordinary day of military service. For the stress examination, groups of four subjects were equipped with an ambulatory electrocardiogram (ECG). Immediately after the collection of a saliva sample and mood rating, subjects were introduced to the upcoming stress task. After completion of the TSST-G, subjects were asked to deliver a second saliva sample and to complete another mood questionnaire. Afterwards, subjects removed the ambulatory device and were allowed to leave. After the second examination the TSST-G committee debriefed participants, explaining the intentions of the test and the two-part study.

Stress Provocation

To induce acute psychosocial stress, a slightly adapted version of the Trier Social Stress Test for Groups (TSST-G; von Dawans et al., 2011) was applied. The TSST-G constitutes a standardized motivated performance task protocol that combines high levels of social-evaluative threat and uncontrollability in a group setting, as used in the original version of the TSST (Kirschbaum et al., 1993). In the present study, the four participants sat next to each other, separated by partitions. After a baseline period (2 min), subjects were introduced to the upcoming task and allowed to prepare themselves (2 min). Then the first subtest began with a fake expert panel (a woman and a man) entering the room and turning on two video cameras, to make the subjects believe they were being videoed. Each subject was required to speak (mock job interview) for about 2 minutes until the experts interrupted them and requested the next person to speak (duration: 4 x 2 min = 8 min). After completion of the fourth short interview, participants were asked to perform a 2-minute mental arithmetic task consisting of a continuous subtraction, to be conducted as fast and accurately as possible. When a mistake occurred, subjects were required to start over from the beginning (Kirschbaum et al., 1993) (duration: 4 x 2 min = 8 min). After both tasks were completed, the expert panel turned off the video cameras and left the room.

During the second exposure the subtasks of the TSST-G were slightly modified to avoid the possibility that recruits would present a memorized speech or to avoid recognition effects of the mental arithmetic task. For the first subtask, two different speech protocols were applied: One subgroup of recruits (TSST-G-2nd-defence) was asked to imagine that they were falsely accused of shoplifting a belt and were required to make a speech in their defence (Saab et al., 1989; Burleson et al., 2003). The other subgroup (TSST-G-2nd-presentation) was given a short article about domestic politics, foreign policy, or economics and told that they would have two minutes to read the articles and prepare a speech about the given topic (adapted from Al'Absi et al., 1997). For the mental arithmetic subtask, the initial number of the serial subtraction task was changed in the second session.

Sociodemographics, Anthropometrics, and Affect Questionnaire

In addition to a short questionnaire on sociodemographic variables (age, education, smoking status) and the measurement of anthropometrical data (height and weight) using a portable stadiometer (model 213; Seca, Hamburg, Germany) and a calibrated scale (Seca model 861), the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) was distributed immediately before and after the TSST-G. The PANAS consists of 10 items for positive affect (e.g. fun) and 10 items for negative affect (e.g. ashamed), which can be answered on a 5-point Likert scale (from 1 = "very slightly or not at all," to 5 = "extremely"). Ratings were summed to a positive and a negative affect score.

Salivary Alpha-Amylase

Saliva samples were collected concomitantly while the participants were filling out the PANAS, immediately before and after the TSST-G. Participants were requested to gently chew a Salivette (Sarstedt, Sevelen, Switzerland) for one minute. The samples were stored at -20^{0}C. After thawing, the biochemical analyses were conducted in the biochemical laboratory of the Clinical Psychology and Psychotherapy department at the University of Zurich, Switzerland. The activity of salivary alpha-amylase (sAA) was analyzed with a kinetic colorimetric test using assay kits (Roche 11555685 a-Amylase Liquid acc) and the automatic analyzer (Biotek Instruments, Lucern, Switzerland) with software KC4 (Roche, Basel, Switzerland).

Electrophysiological Measures

Cardiac activity was continuously measured using an ambulatory electrocardiogram (ECG; Equivital System; Hidalgo, Cambridge, UK), consisting of a chest belt with three embedded electrodes. ECG data was imported, edited, and analyzed using the VivoSense software (Vivonoetics, US). Participants sat in an upright position in order to minimize motion artifacts in the ECG signal. The record was visually examined for artifacts and edited manually to correct for ectopic beats and arrhythmias by using linear interpolation. After artifact correction, heart rate (HR) and heart rate variability (HRV; i.e. RMSSD: the square root of the mean of the sum of the squares of differences between adjacent normal-to-normal (NN) intervals; a time domain measure based on interval differences, which reflect alterations in autonomic tone that are predominantly vagally mediated) were determined (Sztajzel, 2004) for 2-minute intervals and averaged, where necessary, for the following segments: baseline (1 x 2 min), introduction (2 x 2 min), speech task (4 x 2 min); mental arithmetic task (4 x 2 min) and recovery (1 x 2 min), which took place immediately after completion of the TSST-G (after all 4 participants were finished).

Statistical Analyses

Analyses were performed using SPSS for Macintosh, version 19.0. In addition to raw data, delta values were calculated to explore the difference between two measurements (i.e. subtraction of baseline values from peri- or post-test levels), indicating a stress-induced response.

We used analyses of covariance for repeated measures (ANCOVAs) with TIME (two segments of TSST-G for PANAS and sAA: before, after; five segments of TSST-G for HR and HRV: baseline (BL), introduction (Intro), speech task (S1), mental arithmetic task (S2), recovery (Rec)), EXAMINATION (two measurement points: 1st and 2nd) as repeated factors and CONDITION (defence and presentation) as a between factor. Variables in which the two subgroups significantly differed were included as covariates.

For the two subgroups (defence, presentation), HR and HRV data were analyzed using univariate analysis of variance (ANOVA) with repeated measurement. Paired Student's t-tests were computed to compare the raw and delta values between the first and second examination or between different segments within the same examination. Analyses of covariance for repeated measures (ANCOVA) were performed to reveal possible EXAMINATION and TIME effects after Greenhouse-Geisser adjustment. With regard to the main effects of TIME and EXAMINATION x TIME interactions, the effect size was determined by partial eta-square (partial eta^2) reflecting small (.01), medium (.06) or large (.14) effect sizes (Green et al., 2000). All analyses were two-tailed, with a level of significance of $p < .05$.

RESULTS

Participant Characteristics

In order to conduct a large number of psychobiological examinations in the time window provided by the army, the duration of each examination was restricted. Unfortunately, some participants did not manage to complete the mood questionnaires (PANAS) in the time available, reducing the sample size (PA = positive affect, NA = negative affect; this results in: $n_{PA,1st} = 107$, $n_{NA,1st} = 105$; $n_{PA,2nd} = 82$, $n_{NA,2nd} = 80$); with regard to sAA, some samples could not be analyzed due to an insufficient amount of saliva (resulting in: $n_{sAA,1st} = 294$, $n_{sAA,2nd} = 205$). With regard to the ECG data, some files were lost due to technical problems or poor recording quality (resulting in: $n_{HR,1st} = 261$, $n_{RMSSD,1st} = 261$, $n_{HR,2nd} = 187$, $n_{RMSSD,2nd} = 187$). In addition, 51 recruits quit the service by the second examination due to physical or psychological issues (see Figure 1).

The total sample at the first examination consisted of 294 male recruits with a mean age of 20.31 years (SD = 1.19) and a mean body mass index (BMI) of 23.59 kg/m^2 (SD = 3.09).

38.8% were smokers (as detailed in Table 1). Regarding education level, 30.9% had completed lower secondary school, 37.9% upper secondary school, and 31.2% had been in academic high school (qualification for university entrance).

At the first measurement, the two subgroups (TSST-G-1st of the defence subgroup: $n = 151$; TSST-G-1st of the presentation subgroup: $n = 137$) did not differ with regard to age, height and smoking, but there were differences in BMI ($p = .005$) and education level ($p < .001$). Additionally, the two subgroups differed between the baseline value of sAA ($F(1, 273) = 15.249$, $p < .001$) but not in other baseline values.

First Exposure to the TSST-G

The recruits showed a significant decrease in positive affect ($t(102) = 6.058$, $p < .001$) and a significant increase in negative affect ($t(102) = -5.644$, $p < .001$) in response to the TSST-G-1st. Furthermore, a significant increase in sAA activity ($t(292) = -6.612$, $p < .001$) and HR (main effect of TIME, $F(3.011,746.678) = 67.518$, $p < .001$, partial $\text{eta}^2 = .214$) were observed. In line with expectations, RMSSD decreased (main effect of TIME, $F(3.26, 780.84) = 26.92$, $p < .001$, partial $\text{eta}^2 = .098$) during the TSST-G-1st.

Repeated Exposure to the TSST-G

Positive affect responses

The interaction effect revealed no significant effect with regard to positive affect (EXAMINATION x TIME x CONDITION, $F(1,57) = 3.616$, $p = .062$, partial $\text{eta}^2 = .060$).

TSST-G-2nd-defence revealed no significant effect with regard to positive affect ($t(40) = 1.518$, $p = .137$). The TSST-G-2nd-presentation revealed a significant increase with regard to positive affect ($t(40) = 3.704$, $p = .001$).

In the TSST-G-2nd-defence subgroup, the interaction of EXAMINATION x TIME was not significant for positive affect ($F(1, 27) = .108$, $p = .745$, partial eta$^2 = .004$). But in the TSST-G-2nd-presentation subgroup, the interaction of EXAMINATION x TIME was significant for positive affect ($F(1, 34) = 6.675$, $p = .014$, partial eta$^2 = .164$). T-tests revealed a smaller decrease in positive affect (delta, $t(92) = -2.001$, $p = .048$) during the second exposure (see Figure 2).

When comparing the results of both subgroups during the second exposure, no significant difference was observed in positive affect ($F(1, 76) = .707$, $p = .403$, partial eta$^2 = .132$).

Negative affect responses

The interaction effect revealed no significant effect with regard to negative affect (EXAMINATION x TIME x CONDITION, $F(1,54) = 3.226$, $p = .636$, partial eta$^2 = .004$).

TSST-G-2nd-defence ($t(40) = -.051$, $p = .960$) and TSST-G-2nd-presentation ($t(38) = .862$, $p = .394$) revealed no significant effect with regard to negative affect.

In the TSST-G-2nd-defence subgroup, the interaction of EXAMINATION x TIME was significant for negative affect ($F(1, 25) = 5.866$, $p = .023$, partial eta$^2 = .190$). T-tests revealed a smaller increase in negative affect during the second exposure ($t(63) = 4.155$, $p < .001$). In the TSST-G-2nd-presentation subgroup, the interaction of EXAMINATION x TIME was significant for negative affect ($F(1, 33) = 5.893$, $p = .021$, partial eta$^2 = .152$). T-tests revealed a smaller increase in negative affect (delta, $t(93) = 3.934$, $p < .001$) during the second exposure (see Figure 2).

When comparing the results of both subgroups during the second exposure, no significant difference was observed in negative affect ($F(1, 74) = .202$, $p = .654$, partial eta$^2 = .073$).

Salivary alpha-amylase responses

The interaction effect revealed a significant effect with regard to sAA activity (EXAMINATION x TIME x CONDITION, $F(1,181) = 4.305$, $p = .039$, partial eta^2 = .023).

The TSST-G-2nd-defence elicited a significant increase in sAA activity ($t(103) = -2.128$, $p = .036$) and the TSST-G-2nd-presentation had no significant effect on sAA activity ($t(97) = -1.580$, $p = .117$).

In the TSST-G-2nd-defence subgroup, no significant interaction of EXAMINATION x TIME was found for sAA ($F(1, 100) = .198$, $p = .657$, partial eta^2 = .002). In the TSST-G-2nd-presentation subgroup, the interaction was further significant for sAA ($F(1, 97) = 4.079$, $p = .046$, partial eta^2 = .040), which was due to a smaller increase in sAA (delta, $t(97) = 2.020$, $p = .046$) during the second examination (see Figure 3).

When comparing the results of both subgroups during the second exposure, no significant difference was observed in sAA reactivity ($F(1, 180) = 2.157$, $p = .144$, partial eta2 = .309).

Cardiovascular responses: heart rate

The interaction effect revealed a significant effect with regard to HR (EXAMINATION x TIME x CONDITION, $F(3, 371) = 5.041$, $p = .003$, partial eta^2 = .035).

The TSST-G-2nd-defence ($F(2.87, 249.65) = 51.142$, $p < .001$, partial eta^2 = .370) and the TSST-G-2nd-presentation ($F(3.37, 330.16) = 40.881$, $p < .001$, partial eta^2 = .294) elicited a significant increase in HR.

In the TSST-G-2nd-defence subgroup, t-tests revealed a smaller HR increase from BL to Intro ($t(81) = -3.705$, $p < .001$), no difference for the increase from BL to S1 ($t(81) = -1.313$, $p = .193$) and to S2 ($t(81) = 1.178$, $p = .242$), and a smaller recovery ($t(75) = 2.126$, $p = .037$) during the first examination as compared to the second examination (Figure 4).

In the TSST-G-2nd-presentation subgroup, t-tests revealed a stronger HR increase from BL to Intro ($t(73) = -2.530$, $p = .014$), but a smaller increase from BL to S1 ($t(73) = 2.809$, $p = .006$) and S2 ($t(73) = 3.976$, $p < .001$) during the second as compared to the first examination. Recovery did not differ between the first and second examination ($t(73) = .916$, $p = .363$; Figure 4).

When comparing the results of both subgroups during the second exposure, a significant difference in HR ($F(3.13, 531.45) = 3.251$, $p = .020$, partial eta^2 = .019) was observed. ANCOVA analyses revealed a smaller stress response in the TSST-G-2nd-presentation in comparison to the TSST-G-2nd-defence group: delta values from BL to Intro ($F(1, 170) = 4.361$, $p = .038$, partial eta^2 = .025), from BL to S1 ($F(1, 170) = 8.648$, $p = .004$, partial eta^2 = .048), and from BL to S2 ($F(1, 170) = 5.432$, $p = .021$, partial eta^2 = .031). No significant difference was seen for the delta value from BL to Rec ($F(1, 170) = 2.192$, $p = .141$, partial eta^2 = .013).

Cardiovascular responses: heart rate variability

The interaction effect revealed a significant effect with regard to HRV (EXAMINATION x TIME x CONDITION, $F(3,458) = 3.998$, $p = .006$, partial eta^2 = .028).

TSST-G-2nd-defence elicited a significant decrease in RMSSD ($F(3.21, 278.84) = 10.830$, $p < .001$, partial eta^2 = .111). TSST-G-2nd-presentation also elicited a significant decrease in RMSSD ($F(2.97, 288.29) = 7.840$, $p < .001$, partial eta^2 = .075).

In the TSST-G-2nd-defence subgroup with regard to RMSSD, t-tests revealed significant differences in delta values for changes from BL to Intro ($t(81) = 4.655$, $p < .001$), S1 ($t(81) = 4.078$, $p < .001$), S2 ($t(81) = 3.420$, $p = .001$) and Rec ($t(75) = 3.222$, $p = .002$), indicating a stronger parasympathetic stress response during the second exposition (Figure 5).

In the TSST-G-2nd-presentation subgroup with regard to RMSSD, t-tests comparing the first and second examination revealed no difference with regard to changes between BL to Intro ($t(73) = -.997$, $p = .322$), BL to S1 ($t(73) = -1.426$, $p = .158$), BL to S2 ($t(73) = -1.479$, $p = .144$), and BL to recovery ($t(73) = .079$, $p = .937$) (Figure 5).

When comparing the results of both subgroups during the second exposure, a significant difference in RMSSD ($F(3.24, 548.20) = 4.687$, $p = .002$, partial eta$^2 = .027$) was observed. ANCOVA analyses revealed the results: a smaller change from BL to Intro ($F(1, 169) = 15.336$, $p < .001$, partial eta$^2 = .083$), from BL to S1 ($F(1, 169) = 10.348$, $p = .002$, partial eta$^2 = .058$) and from BL to S2 ($F(1, 169) = 7.894$, $p = .006$, partial eta$^2 = .044$) in TSST-G-2nd-presentation in comparison to TSST-G-2nd-defence. No difference was found for the change from BL to Rec ($F(1, 170) = .523$, $p = .471$, partial eta$^2 = .003$).

DISCUSSION

The present study aimed to investigate the affective and autonomic responses to (a) single and (b) repeated exposure to the TSST-G with an in-between interval of 10 weeks, and (c) comparing the two different subversions of the TSST-G, which were applied during the second exposure, with regard to their efficacy to induce significant psychobiological stress responses in healthy young men.

The first exposure to the TSST-G significantly decreased positive and increased negative affect, increased sAA as well as HR, and decreased RMSSD. During the second examination while neither subversions of the TSST-G affected negative affect, the TSST-G-2nd-presentation (but not the TSST-G-2nd-defence) revealed a decrease in positive affect. In contrast, sAA activity was only significantly increased in TSST-G-2nd-defence, while both subgroups revealed an increase in HR and a decrease in RMSSD. When comparing both subversions during the second exposure, the HR and RMSSD stress response was larger in the TSST-G-2^{nd}-defence subgroup than in the TSST-G-2^{nd}-presentation subgroup. When comparing the first and second examination, the second revealed a smaller response with regard to negative and positive affect. With regard to sAA, one group revealed a reduced response (TSST-G-2^{nd}-presentation), while the other did not. With regard to HR and RMSSD, one group indicated a stronger response (TSST-G-2^{nd}-defence) while the other indicated a smaller response with regard to HR and no change with regard to RMSSD (TSST-G-2^{nd}-presentation).

Participants experienced a noticeable worsening of mood during the first TSST-G exposure. This result replicates findings from two previous studies testing groups of two and three (Childs et al., 2006) or six participants (von Dawans et al., 2011). In addition, during the first examination, sAA activity was significantly increased in response to the TSST-G. In accordance with our results, others confirmed an increased sAA activity in response to the TSST in individual settings (Nater et al., 2006; Schoofs & Wolf, 2011). The present study replicates and extends results by two previous studies, which tested the TSST-G one time (Childs et al., 2006; von Dawans et al., 2011). In line with both previous studies, we found an increase in HR during the TSST-G. In addition, we found a significant decrease in HRV during the TSST-G. Until now, a decrease in HRV during TSST has only been examined during single settings (Nater et al., 2006; Strahler et al., 2010). Our results suggest that the TSST-G induces similar effects regarding psychological, sympathetic nervous system, and parasympathetic nervous system variables as the original single-subject version (e.g. Kirschbaum et al., 1993; Nater et al., 2006; Strahler et al., 2010).

A worsening of mood was partially confirmed during the second exposure (i.e. for the TSST-G-2^{nd}-presentation group). With regard to the effect of repetition on affect ratings, no references exist for group examinations. Therefore, in this section citations refer to studies of TSST in single settings. Cohen et al. (2000) showed that fear and stress levels increased during a second exposure to the TSST (two week interval), but the increases were less extreme as during the first exposure. Similarly, Jönsson et al. (2010) found that subjects felt significantly less frightened during a second exposure to the TSST (one week interval). These results are in line with our findings, in which we detected a partial habituation in positive and negative affect during a repeated exposure to the TSST-G. The TSST-G-2nd-presentation induced a significant decrease in positive affect whereas the TSST-G-2nd-defence did not. It should be noted that even during the first exposure to the TSST-G, the TSST-G-2nd-presentation subgroup revealed a stronger decrease in positive affect than the TSST-G-2nd-defence subgroup. This indicates a stronger affective stress sensitivity of this group. In accordance with our findings from the TSST-G-2nd-defence group, previous measurements of sAA revealed no habituation to repeated application (Wüst et al., 2005; von Känel et al., 2006). Nevertheless, we observed a reduced response in the subversion with the TSST-G-2nd-presentation, suggesting that the defensive speech was experienced as more stressful. It is important to mention that the response to the first examination of the presentation subgroup was higher compared to the response of the defence subgroup. Reasons for this are somewhat speculative and might be explained by differences in the military schedule or demands during the first week of the basic military training. During the second exposure to the TSST-G, a significant increase in HR was detected. Previous studies about the repeated application of the TSST in single settings showed, similarly, no habituation with regard to the HR response (Gerra et al., 2001; von Känel et al., 2006; Jönsson et al., 2010) or a minimal decrease over time (Schommer et al., 2003). In the present study, the repeated application showed no (TSST-G-2nd-defence) or a slight (TSST-G-2nd-presentation) habituation with regard to HR. Taken together, these results support the effectiveness of the TSST-G in repeated applications to induce a stress response of HR.

In line with the HR results of the present study, a significant decrease in HRV during the TSST-G examinations was found. Petrowski and colleagues (2012) did not notice any habituation of HRV in response to repeated TSST challenges in a single setting. Similarly, in our case there was not a remarkable habituation in the TSST-G-2nd-presentation subgroup concerning HRV. However, the TSST-G-2nd-defence subgroup revealed a significant increase in the HRV response, suggesting that this kind of stress task seems to provoke a stronger parasympathetic inhibition during the second exposure.

The different response patterns of both subgroups during the second examination illustrated that a stress task consisting of reading and presenting a speech about a given topic is less stressful than a task requiring a defensive speech in response to a false shoplifting accusation. This result is supported by the fact that the autonomic stress response in the TSST-G-2nd-presentation condition was weaker than in the TSST-G-2nd-defence condition. This might be explained by differences in ego-involvement. Mason (1968) reported that psychological stress is caused by factors such as novelty and uncontrollability in combination with ego-involvement. A speech on an impersonal topic probably involves less ego than a fake accusation followed by a personal defence. Similar to the job interview, during the defence condition the participant constitutes the center of focus. Whereas in the given topic conditions, the article provided by the researchers and the subsequent presentation constitute the focus of attention. Therefore, it is possible for the participant to maintain a certain distance; hence the stress response is more moderate.

Meanwhile, we observed greater changes in HRV between the first and second measure in the TSST-G-defence group. The main difference between the job interview and the defence scenario was the fact of highlighting a positive personal impression during the job interview, while during the defensive speech identification with an anti-social behavior was required.

This difference might be responsible for the bigger response in HRV during the defence speech than during the job interview.

Compared to the first exposure, both subgroups showed a stronger increase in HR from baseline to the introduction. Therefore, the anticipatory stress response was stronger during the second exposure. The importance of anticipation of negative consequences is well known (Mason, 1968). This adaption is supported by the fact that the baseline of positive affect decreased significantly from the first to the second examination. As a result, the recruits were feeling worse, even before the second stress task started. During the stress task (speech and arithmetic task) only one subgroup (TSST-G-2nd-defence) showed a similar HR reaction when comparing the first and second examination, while the HR response of the other group (TSST-G-2nd-presentation) decreased from the first to the second examination.

A possible explanation for the worsening of mood from the first to the second examination could be the fact that the recruits felt more stressed or exhausted in general after 10 weeks of training. The recruits could perceive basic military training as a chronic stressor (Hellhammer et al., 1997; Martin et al., 2006). In addition, we found a dissociation between psychological and biological responses (see Campbell and Ehlert, 2012).

Despite the interesting findings, this study contains a number of limitations. Saliva and affective ratings were not measured immediately after individual performance, but after all four participants had finished the TSST-G. Therefore, stronger effects can be assumed with data collection immediately after individual performance of the subtasks. Sequence effects can be assumed, with differences in stress response with regard to whether a subject is currently speaking or recovering from his turn. Therefore, it can be assumed that the magnitude of response was influenced by the order of participation, constituting an inevitable problem in the application of the TSST-G. The chosen affect questionnaire (PANAS), with

20 items, was obviously too long for the short timeframe provided in the military setting. This led to a large number of missing questionnaires (about 50%). Visual analogue scales might have been a better alternative for the military setting.

Another point of criticism is the fact that the subgroups could not be randomized because of a military allocation to different companies. The lack of randomization might have contributed to the group differences at the beginning. The presented data must, therefore, be interpreted with caution. Another limitation worth mentioning concerns generalization, since subjects were taken out of their usual environment and went through a period of chronic physiological and psychological stress. A further point of limitation is that the social evaluation during basic military training could be higher because recruits know each other at least by sight. Furthermore, the present findings cannot be generalized to women or other age groups, since only young men were examined. But this sample homogeneity can also be considered as an advantage of the study, because possible influencing factors like age and gender can be excluded. Despite the mentioned limitations, the present study has several strengths. To our knowledge, this is the first study to measure sAA activity and HRV in response to the TSST-G. In addition, no other study to date has examined the effects of repeated application of the TSST-G. Another strength of the present study is the fact that two different adaptations of the repeated TSST-G were applied to determine which version would be more effective.

It would be interesting to assess salivary cortisol measurements in response to repeated exposure to the TSST-G. In our present study, this was not possible due to time constraints. The HPA and ANS are two different strategies of the organisms to deal with stress (Gerra et al., 2001). The group version of the TSST — involving a larger social-evaluative threat — could help ensure that subjects habituate less with regard to the HPA than in the single version. Therefore, the TSST-G might be more effective for repeated stress induction than the TSST. In future studies, it would be of further interest to measure sequence effects regarding the order of speakers. This could possibly influence the course of continuously or repeatedly collected data (e.g. HR or sAA). Furthermore, due to the fact that the presented data is exploratory, it is necessary to test the corresponding hypotheses and results in further confirmatory studies.

In summary, our data suggest that the TSST-G is a useful protocol to evoke a stress response of the autonomic nervous system and affect. Furthermore the TSST-G is useful to evoke repeated stress responses, even though habituation is evident especially with regard to affect. When interested in repeated exposure, for the second examination a subversion with a topic concerning the subject itself (TSST-G-2nd-defence) seems more promising in inducing a significant stress response than a speech on an impersonal topic (TSST-G-2nd-presentation). We can recommend the TSST-G as an economical stress test for laboratory settings, especially for studies interested in the examination of large samples and in settings with time limitations.

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Figures

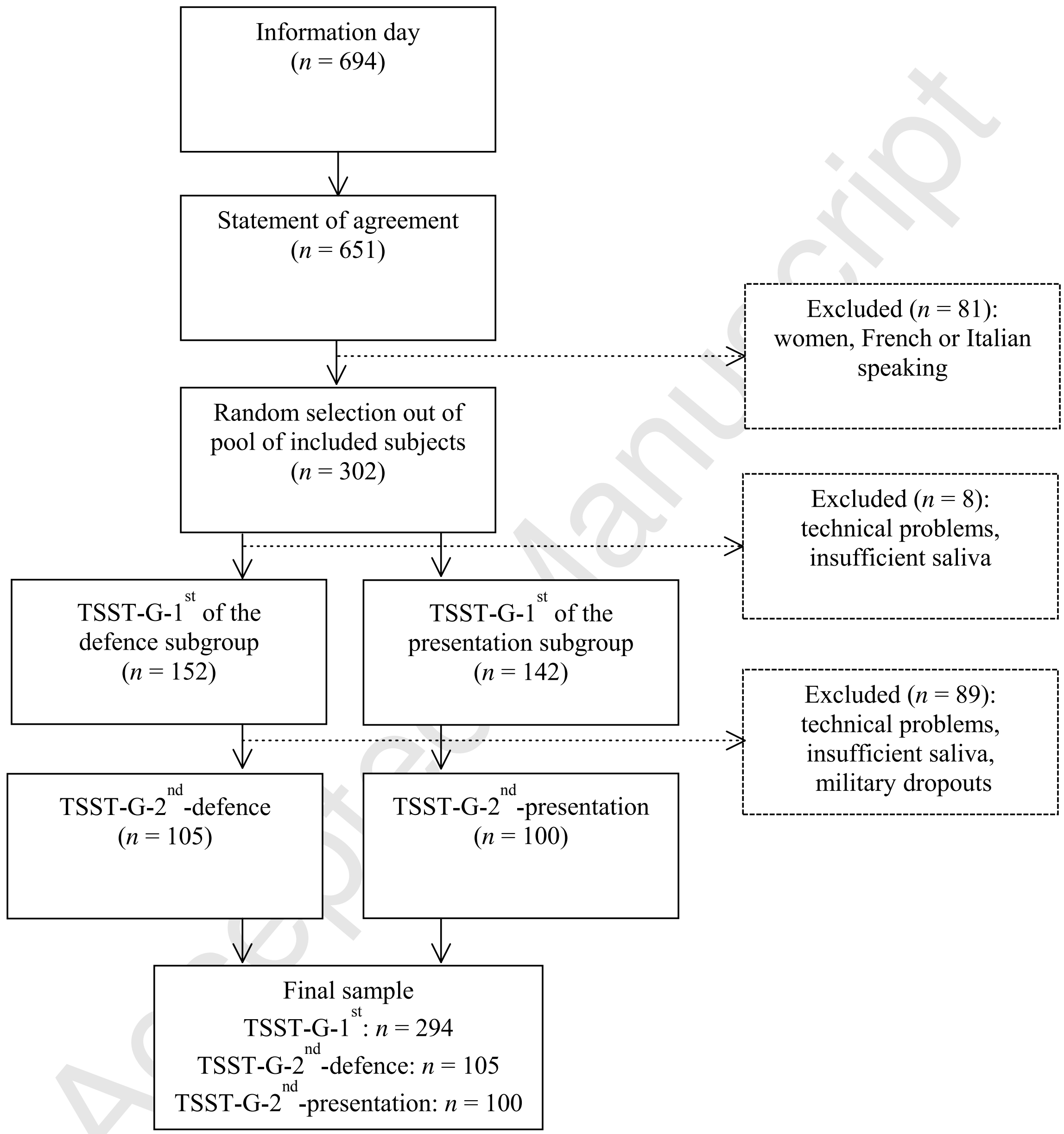

Figure 1: Flow chart of the selection and failures of participants. Two examinations (1st and 2nd) were conducted. During the first examination all subjects participated in the original TSST-G version; for the second two slightly different TSST-G protocols were applied (TSST-G-2nd-defence and TSST-G-2nd-presentation) in two sample subgroups.

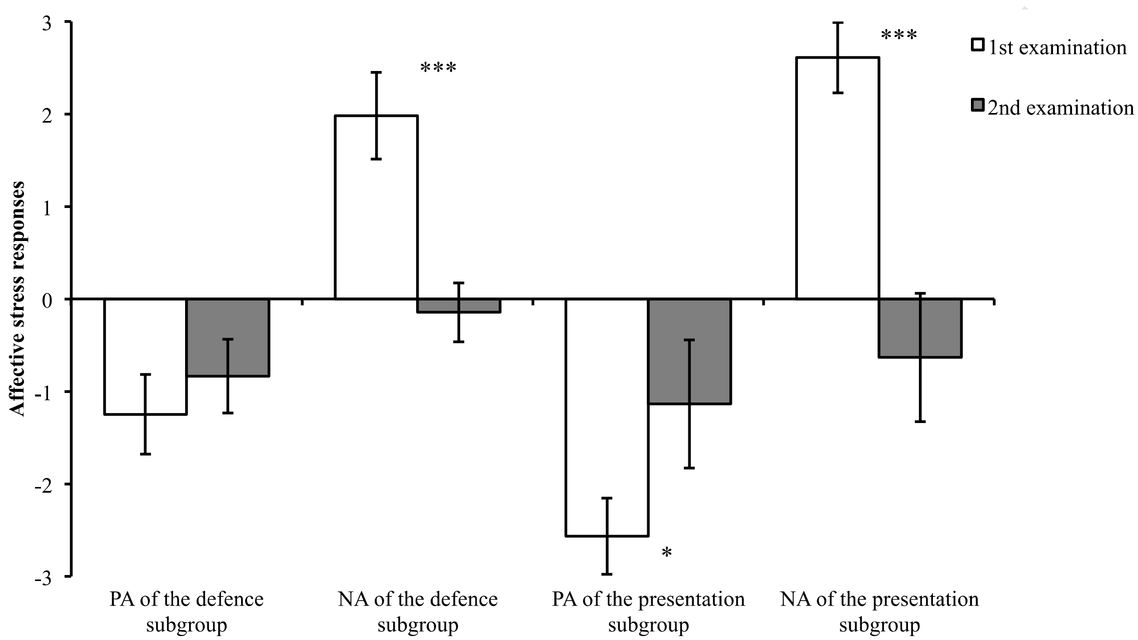

Figure 2: Stress response of positive (PA) and negative affect (NA) during the 1st and 2nd examination of TSST-G-defence and TSST-G-presentation. Data are presented in delta values ± SEM (standard error of mean), **p* < .05, ****p* < .001.

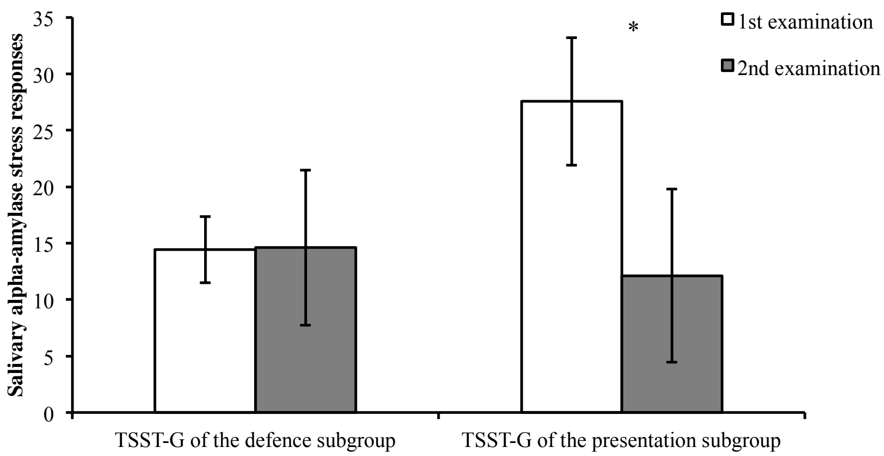

Figure 3: Stress response of salivary alpha-amylase (sAA; U/ml) during the 1st and 2nd examination of TSST-G-defence and TSST-G-presentation. Data are presented in delta values ± SEM (standard error of mean), *$p < .05$.

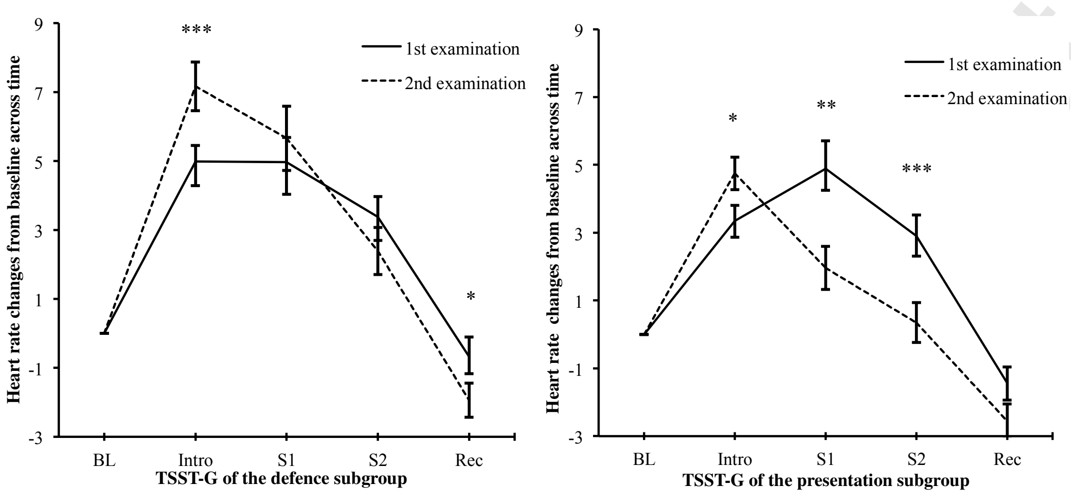

Figure 4: Stress response of heart rate (bpm) during the 1st and 2nd examination of TSST-G-defence and TSST-G-presentation (BL: baseline; Intro: introduction; S1: speech task; S2: mental arithmetic task; Rec: recovery). The statistical results are identical to the results of the repeated measures ANOVA with the absolute values; mean ± SEM (standard error of mean), **p* < .05, ***p* < .01, ****p* < .001.

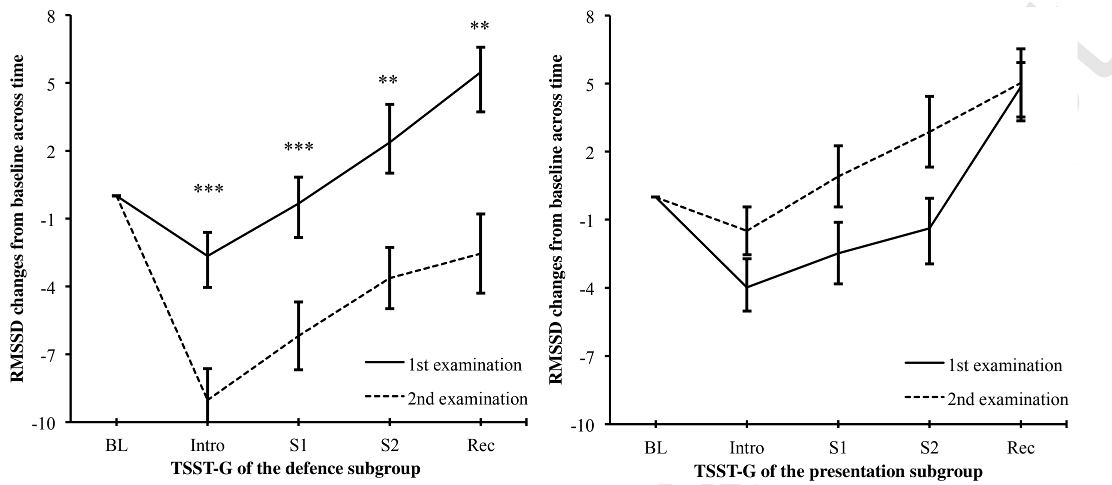

Figure 5: Stress response of RMSSD during the 1st and 2nd examination of TSST-G-defence and TSST-G-presentation (BL: baseline; Intro: introduction; S1: speech task; S2: mental arithmetic task; Rec: recovery). The statistical results are identical to the results of the repeated measures ANOVA with the absolute values; mean±SEM (standard error of mean), ***p* < .01, ****p* < .001.

Tables

Sociodemographics	TSST-G-1st of the defence subgroup (*n* = 152)	TSST-G-1st of the presentation subgroup (*n* = 142)
Age (years)	20.18 ± 1.34	20.46 ± 0.97
Education		
Lower Secondary School	23.5%	39.0%
Upper Secondary School	31.5%	44.9%
Academic High School	45.0%	16.1%
Height (cm)	177.51 ± 6.89	177.81 ± 6.41
Weight (kg)	72.85 ± 10.08	76.38 ± 11.90
Body mass index (kg/m^2)	23.11 ± 2.87	24.12 ± 3.26
Smoker	33.5%	44.4%

Table 1: Descriptive information collected at the first examination, for participants of both subgroups (defence and presentation). Values are expressed in means ± standard deviation or percentage.